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Use of transmission electron microscope to assess the damage to Sarcoma 180 ascites tumour cells following *in vivo* treatment of mitomycin-C and gamma radiation

Sandip Kumar Majumdar, Somenath Bhattacharya, Aruna Mitra and Sheela Mukherji

Biophysics Department, Chittaranjan National Cancer Research Centre, 37, S P Mukherjee Road, Calcutta-700 026, India

Abstract: Five day old Sarcoma 180 tumour bearing mice were exposed to different doses of mitomycin-C (4 mg or 7 mg per kg body weight of mouse) and gamma radiation (400 R or 800 R) applied singly or in combination. Surviving populations were collected after 5 days of treatment and processed for transmission electron microscopy. The control Sarcoma 180 tumour cells has the following characteristics; profused microvilli, different sized mitochondria with poorly developed internal structure, distinct endoplasmic reticulum studded with ribosomes, the large nucleus rich in chromatin materials and distinct nucleolus containing closely interwind granular and fibrillar components with associated chromatins. Damage to treated cells were ascertained by the reduction in microvilli, swelling of mitochondria with cloudy appearance, dilation and fragmentation of endoplasmic reticulum, blebbing of nuclear membrane, condensation of heterochromatin, appearance of perichromatin granules, segregation and fragmentation of nucleolus and invagination of plasma membrane with increased intracellular spaces. With the help of transmission electron microscope it is thus possible to assess the nature of damage to organelles effected by mitomycin-C and radiation both singly and in combination. Growth inhibition and damage in the cellular ultrastructure were maximum among tumour cells which survived with concomitant treatment with 7 mg MMC and 800 R.

Keywords: Sarcoma 180, mitomycin-C, gamma radiation, tumour growth, ultrastructure.

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1. Introduction

The role of transmission electron microscope as an useful physical technique in medical sciences is well documented (Carr and Toner 1968, Ghadially 1975, 1985). Because of its extraordinary resolving power (De Robertis and De Robertis 1980), the electron microscope seems to be an ideal instrument for the study of cellular ultrastructure. The purpose of the present study was to investigate whether changes in subcellular organelles of Sarcoma 180 tumour cells could be distingui-

shed electron microscopically when mitomycin-C (MMC, an antitumour antibiotic) and gamma radiation were applied singly or in combination on Swiss mice bearing this neoplasm in ascites form.

2. Materials and methods

Sarcoma 180 ascites tumour was maintained in the laboratory through serial intraperitoneal transplantation (10^7 cells per Swiss albino mice, av. wt. 20-22 g, 8-10 weeks old). After five days of tumour transplantation the animals were divided into four groups. Group A of animals was kept as control, Group B of animals was given a single i.p. injection of MMC in 0.01 M phosphate buffer (pH 7.2) in a dose of 4 mg or 7 mg per kg body weight of mouse. Group C of animals was whole body irradiated with 400 R or 800 R at a dose rate of 62 R/minute (^{137}Cs Source, Picker USA, at Cancer Hospital, Calcutta). Group D of animals received MMC and gamma radiation concomitantly. The viable number of tumour cells per ml of ascitic fluid in both control and treated groups were measured after 10 days of transplantation (i.e. 5 days after treatment) using trypan blue dye exclusion test. The percentage growth inhibition was determined (Matsumoto *et al* 1986) from the relation $(1-\frac{T}{C})\times 100$, where T and C were the viable cell numbers of treated and control groups respectively.

For transmission electron microscopy the tumour cells were collected from control and treated group of animals on the same day and washed with normal saline. The cells were fixed in phosphate buffered (pH 7.2) paraformaldehyde-glutaraldehyde mixture (1.5% : 1%) at room temperature for 30 mins. The cells were rinsed successively in phosphate buffer and veronal acetate (VAC) buffer (pH 7.2) and post-fixed in 1% osmium tetroxide (buffered with veronal acetate) for 18-22 hrs. in dark at room temperature. Following another rinse with VAC buffer, the cells were stained in 0.5% uranyl acetate enblock for 90 mins at room temperature and dehydrated in a graded series (30 to 100%) of alcohol. The samples were infiltrated and embedded in low viscosity epoxy resin (Spurr 1969). Ultrathin sections cut with an ultramicrotome (NOVA, LKB) and stained with lead citrate were examined in Hitachi H-600 transmission electron microscope at an accelerating voltage of 75 KV.

3. Results

The growth of Sarcoma 180 tumour cells was inhibited when they are subjected to different treatment modalities. Table 1 shows that the percentage growth inhibition increased with increased dose of MMC and radiation. While MMC treatment alone inhibited the tumour growth from 35 to 37%, in combination with radiation its effectivity was increased significantly (47 to 56%). Whole body radiation reduced the growth to an extent of 13 to 22% only. Thus tumoricidal activity of MMC enhanced considerably when it was applied concomitantly with gamma radiation on Sarcoma 180 ascites tumour (Majumdar and Mukherji 1987).

Transmission electron microscopy was carried out with tumour cells which survived after 10 days of transplantation following 5 days of treatment. Since the maximum growth inhibition occurred in 7 mg MMC plus 800 R treatment,

Table I. *In vivo* effect of mitomycin-C and gamma radiation on growth of Sarcoma-180 tumour cells.

Treatment modality	Viable cell number per ml of ascitic fluid ($\times 10^{-7}$)	Per cent growth inhibition
Untreated control	10.94 ± 2.24	—
400 R	9.50 ± 1.13	13.16
800 R	8.44 ± 1 36	22.85
4 mg MMC	7.03 ± 2.92	35.74
7 mg MMC	6.89 ± 1.47	37.02
4 mg MMC + 400 R	5.80 ± 1.08	47.00
7 mg MMC + 400 R	5.62 ± 0.31	48.60
4 mg MMC + 800 R	4.86 ± 2.32	55.60
7 mg MMC + 800 R	4.80 ± 1.35	56.10

Figures 1-4 represent the ultrastructures of untreated control cell (Figure 1) and those of cells treated with 800 R (Figure 2), 7 mg MMC (Figure 3) and 7 mg MMC+800 R (Figure 4) respectively. The characteristic features of untreated

Figure 1. Untreated Sarcoma-180 ascites cells after 10 days of transplantation. X16,800.

cells are : different sized mitochondria (M) with poorly developed cristae, distinct endoplasmic reticulum (ER) studded with ribosomes, chromatin rich nucleus containing closely interwind granular and fibrillar components in nucleolus (N). Besides these characteristics the untreated cells possess profused microvilli (not

shown in figure). In the 800 R irradiated cells (Figure 2) microvilli are broken (arrow heads), mitochondria are scattered, endoplasmic reticulum is fragmented

Figure 2. Sarcoma-180 ascites tumour cells from whole body irradiated mouse of 800 R after 5 days. X9,450.

(arrows). While nuclear membrane is irregular with foldings, chromatin materials condense at the nuclear periphery with matrix becoming less dense. By treatment with 7 mg MMC (Figure 3) the number of microvilli reduces (arrow heads), the

Figure 3. Sarcoma-180 ascites tumour cells from MMC treated mouse after 5 days. Intraperitoneal injection of MMC at the dose of 7 mg per kg body weight of mouse on the 5th day of transplantation. X18,400.

mitochondria (M) get swollen with cloudy appearance, endoplasmic reticulum becomes scanty. While the nuclear membrane becomes irregular with bleb

formation and deep indentation, heterochromatin materials condense at the periphery (arrows). While at low dose of MMC (4 mg MMC, not shown in figure) nucleolus becomes segregated in two zones viz., dark and light, at higher concentration (7 mg MMC) nucleolus becomes devoid of material (Figure 3). Also found few perichromatin granules (PCG) in the nucleus and intracytoplasmic lumen associated with villi (A) in MMC treated cells.

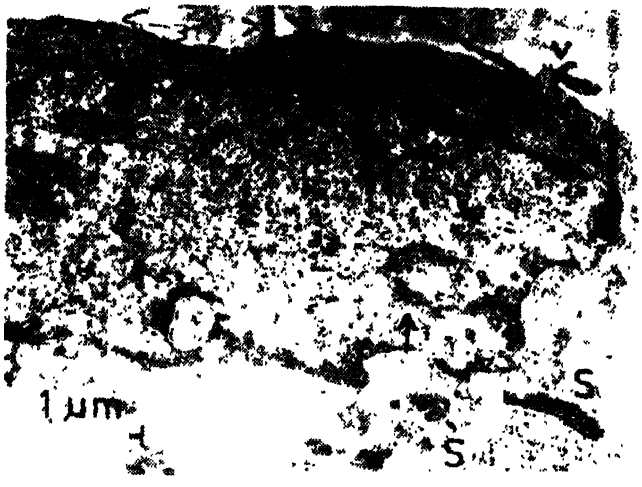

Figure 4. Sarcoma-180 ascites tumour cells after 5 days of *in vivo* treatment with MMC and gamma radiation concomitantly. 7 mg MMC+800 R X19,200.

Major effects of combined treatment with MMC and radiation (Figure 4) are on the nucleolus which becomes fragmented. Electron dense granules shown by arrows in Figure 4 may represent remnant parts of the nucleolus. While reduction in the number of microvilli (arrow heads) is drastic, plasma membrane invaginates increasing intracellular spaces (S)—a characteristic which is never found with single treatment of either MMC or gamma radiation.

4. Conclusion

While increase in tumour growth inhibition is a measure of increase in cytotoxicity, the changes in the major ultrastructural features of the treated cells with respect to untreated tumour indicate the extent of cellular damage brought by any single or combined treatment of MMC and gamma radiation.

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